United States Environmental Protection Agency | Office of Solid Waste and Emergency Response | EPA/540/2-91/013B July 1991

Guide for Conducting Treatability Studies under CERCLA: Aerobic Biodegradation Remedy Screening

Office of Emergency Response and Remedial Response
Hazardous Site Control Division OS-220

QUICK REFERENCE FACT SHEET

Section 121(b) of CERCLA mandates EPA to select remedies that "utilize permanent solutions and alternative treatment technologies or resource recovery technologies to the maximum extent practicable" and to prefer remedial actions in which treatment "permanently and significantly reduces the volume, toxicity, or mobility of hazardous substances, pollutants, and contaminants is a principal element." Treatability studies provide data to support remedy selection and implementation. They should be performed as soon as it becomes evident that the available information is insufficient to ensure the quality of the decision. Conducting treatability studies early in the remedial investigation/feasibility study (RI/FS) process should reduce uncertainties associated with selecting the remedy and should provide a sound basis for the Record of Decision (ROD). Regional planning should factor in the time and resources required for these studies.

This fact sheet provides a summary of information to facilitate the planning and execution of aerobic biodegradation remedy screening treatability studies in support of the RI/FS and the remedial design/remedial action (RD/RA) processes. This fact sheet follows the organization of the "Guide for Conducting Treatability Studies Under CERCLA: Aerobic Biodegradation Remedy Screening, Interim Guidance," EPA/540/2-91/013A, July 1991. Detailed information on designing and implementing remedy screening and remedy selection treatability studies for aerobic biodegradation is provided in the guidance document. This guidance discusses only screening of biological treatment. Remedy selection guidance for aerobic biodegradation is currently in the planning stages.

INTRODUCTION

There are three levels or tiers of treatability studies: remedy screening, remedy selection and remedy design. The "Guide for Conducting Treatability Studies Under CERCLA: Aerobic Biodegradation Remedy Screening" discusses only the remedy screening level.

Remedy screening studies are designed to provide a quick and relatively inexpensive indication of whether biological degradation is a potentially viable remedial technology. Remedy selection and remedy design studies will also be required to determine if bioremediation is a viable treatment alternative for a site. The remedy screening evaluation should provide a preliminary indication that reductions in contaminant concentrations are due to biodegradation and not abiotic processes such as photo decomposition or volatilization. It will also produce the design information required for the next level of testing, should the laboratory screening evaluation be successful. Aerobic biological remedy screening study should not be the only level of technology screening performed before final remedy selection.

TECHNOLOGY DESCRIPTION AND PRELIMINARY SCREENING

Technology Description

Bioremediation generally refers to the breakdown of organic compounds (contaminants) by micro-organisms. In situ, solid-phase, slurry-phase, soil heaping and composting biological treatment techniques are available for the remediation of contaminated soils. Aerobic biodegradation can be used as the only treatment technology at a site or along with other technologies in a treatment train. Use of aerobic biodegradation, especially in situ, has been limited at CERCLA sites. However, the technology shows promise for degrading, immobilizing or transforming a large number of organic compounds commonly found at CERCLA sites to environmentally acceptable compounds.

As of fiscal year 1989 (FY89), in situ biodegradation has been selected as a component of the remedy for 22 Superfund sites having groundwater, soils, sludges, or sediments contaminated with various volatile organics; phenols; creosotes;

polynuclear aromatic hydrocarbons (PAHs); and benzene, toluene, ethyl benzene, and xylene (BTEX) compounds.

The determination of the need for and the appropriate level of treatability studies required is dependent on the literature information available on the technology, expert technical judgement, and site-specific factors. Several reports and electronic data bases exist which should be consulted to assist in planning and conducting treatability studies as well as help prescreen bioremediation for use at a specific site. Site-specific technical assistance is provided to Regional Project Managers (RPMs) and On-Scene Coordinators (OSCs) by the Technical Support Project (TSP).

Prescreening Characteristics

One of the major parameters that influence the feasibility of using biological processes is the biodegradability of the compounds of concern. Prior to conducting a remedy screening of bioremediation it is important to confirm that the compounds of concern are indeed amenable to biological treatment. Consultation with experts and the TSP is critical at this stage.

A literature search should be performed for the compounds of wastes of interest, including compounds of similar structure. The literature review should not be limited to a biodegradation technology which has been chosen for preliminary consideration. The key question to be answered is whether any evidence of aerobic biodegradation of these compounds or wastes exist.

The literature search should also investigate the chemical and physical properties of the contaminants. The volatility of the contaminants is one of the most important physical characteristics. Knowledge of the contaminant volatility is important in the prescreening step since highly volatile contaminants may be volatilized, especially in stirred or highly-aerated reactors, before biodegradation can proceed.

There is no steadfast rule which specifies when to proceed with laboratory screening and when to eliminate aerobic biodegradation as a treatment technology based on a preliminary screening analysis. A literature search indicating that biodegradation is unlikely should not automatically eliminate aerobic biological technologies from consideration. On the other hand, previous studies indicating that pure chemicals will be degraded must be viewed with caution. Chemical interactions or inhibitory effects of contaminants can alter the biodegradability of chemicals in complex mixtures frequently found at Superfund sites. An analysis of the existing literature coupled with the site characterization will provide the information required to make an “educated decision”. However, when in doubt, a laboratory screening study is recommended.

Examples of classes of compounds which are readily amenable to bioremediation are: petroleum hydrocarbons such as gasoline and diesel; wood treating wastes such as creosote and pentachlorophenol; solvents such as acetone, ketones and alcohols; and aromatic compounds such as benzene, toluene, xylenes, and phenols. Several documents/review articles which present detailed information on the biodegradability of compounds are listed in the reference section of the complete guidance document. However, discretion should be exercised when using these reference materials, as micro-organisms which can biodegrade compounds which have traditionally been considered non-biodegradable are continually being isolated through ongoing research and development efforts.

Technology Limitations

Many factors impact the feasibility of aerobic biodegradation in addition to the inherent biodegradability as measured in the screening test. These factors should be addressed prior to the selection of aerobic biodegradation, and prior to the investment of time and funds in further testing. A more detailed discussion of these factors is presented in the guidance document.

THE USE OF TREATABILITY STUDIES IN REMEDY EVALUATION

Treatability studies should be performed in a systematic fashion to ensure that the data generated can support the remedy evaluation and implementation process. A well-designed treatability study can significantly reduce the overall unceratinty associated with the decision, but cannot guarantee that the chosen alternative will be completely successful. Care must be exercised to ensure that the treatability study is representative of the treatment as it will be employed (e.g., the sample is representative of waste to be treated) to minimize the uncertainty in the decision. The method presented below provides a resource-effective means for evaluating one or more technologies.

There are three levels or tiers of treatability studies: remedy screening, remedy selection and remedy design. Some or all of the levels may be needed on a case-by-case basis. The need for and the level of treatability testing required are management decisions in which the time and cost necessary to perform the testing are balanced against the risks inherent in the decision (e.g., selection of an inappropriate treatment alternative). Figure 1 shows the relationship of three levels of treatability study to each other and to the RI/FS process.

Remedy Screening

Remedy Screening is the first level of treatability testing for aerobic biological technologies. It is used to establish the validity of a technology to treat a particular contaminant. These studies are generally low cost (e.g., $10,000-$50,000) and usually require 1 week to several months to complete. They yield data that can be used as a preliminary indication of a technology's potential to meet performance goals and can identify operating standards for investigation during remedy selection testing. They generate little, if any, design or cost data and should not be used as the sole basis for selection of a remedy.

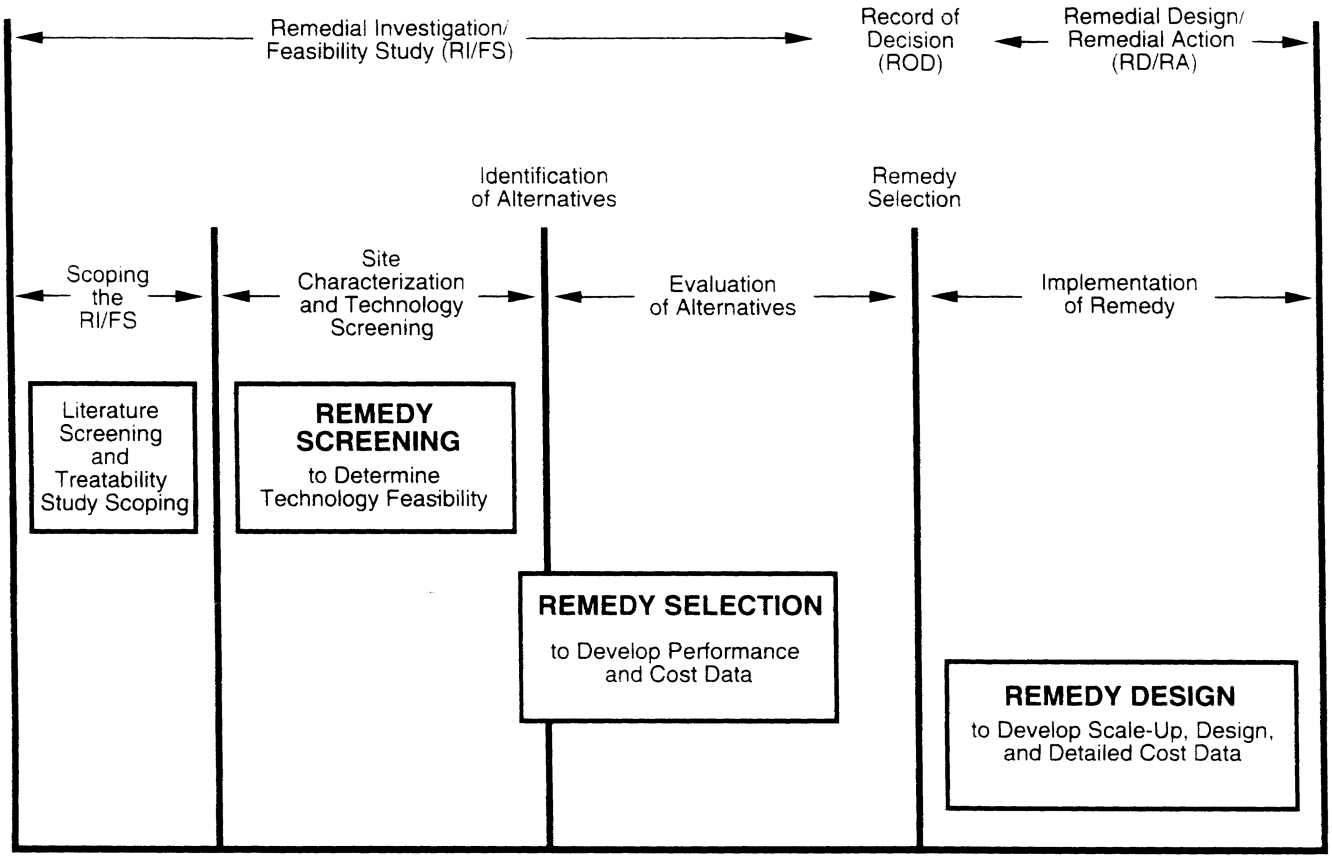


Figure 1. The Role of Treatability Studies in the RI/FS and RD/RA Process

Typically, laboratory-scale aerobic biological screening studies are performed in test reactors provided with sufficient nutrients and oxygen. These reactors may be small sacrificial batch reactors (approximately 40 ml to one liter in size) or larger ecosystems (1 to 10 liters) which are subsampled to monitor the progress of biodegradation. The reactors may contain saturated or unsaturated soil or slurries in water. Normally, pH and contaminant loading rates are adjusted to increase the chances of success. The microbial population can be indigenous to the site, from another acclimated source (i.e., wastewater treatment sludge or another area on site), selectively cultured, a proprietary mixture provided by a vendor, or any combination of the above. The bioreactors are set up for replicate sampling at several time points. The test reactors are compared to inhibited controls at each time point to determine if aerobic biological degradation occurred. The inhibited reactors are treated with sterilization agents in an effort to reduce or eliminate the biological activity in the control reactors. The mean contaminant concentration in the inhibited control replicates is subtracted from the mean contaminant concentration in the test reactors. The goal for a successful treatability test is a removal rate, due to biological processes, which is greater than the analytical error inherent in the test design. A reduction of the contaminant concentration over a three to six week period of 20% (minimum) to 50% or 60% (corrected for non-biological losses) would be typical. The goals of remedy screening are discussed below.

REMEDY SCREENING TREATABILITY STUDY WORK PLAN

Carefully planned treatability studies are necessary to ensure that the data generated are useful for evaluating the validity or performance of a technology. The Work Plan, which is prepared by the contractor when the Work Assignment is in place, sets forth the contractor's proposed technical approach for completing the tasks outlined in the Work Assignment. It also assigns responsibilities and establishes the project schedule and costs. The Work Plan must be approved by the RPM before initiating subsequent tasks. A suggested organization of the Work Plan is provided in the "Guide for Conducting Treatability Studies Under CERCLA: Aerobic Biodegradation Remedy Screening."

Test Goals

Setting goals for the treatability study is critical to the ultimate usefulness of the data generated. Goals must be defined before the treatability study is performed. Each tier of treatability study needs performance goals appropriate to that tier.

The main goals of the remedy screening evaluation are to:

- Provide an indication that reductions in contaminant concentrations are due to biodegradation and not

abiotic processes such as photodecomposition, volatilization, and adsorption.

- Produce the design information required for the next level of testing, should the screening evaluation be successful.

Normally, the average contaminant concentration should be reduced by at least 20% during a six- to eight-week study, as compared to an inhibited control, to conclude aerobic biodegradation is a potential treatment technology for the site under investigation. The 20% contaminant reduction is a matter of professional judgment, but is designed to maximize the chances of success at the remedy screening tier. The choice of a six- to eight-week study is to provide a consistent endpoint for remedy screening studies. The choice of the remedy screening treatability study goals (time and contaminant reduction) will be site-specific decisions.

Experimental Design

A number of different approaches can be used to conduct the remedy screening test. These range from simple shake flask evaluations to soil pans or soil slurry reactors. The soil may be either saturated or unsaturated, depending on the goals of the study. Soil slurries will optimize mixing and will tend to maximize biological degradation. Such studies will maximize the chances of success at the remedy screening level. Unsaturated soils will often limit mixing and result in slower degradation rates. However, such systems will correlate better with field conditions in many cases and result in better extrapolation to remedy selection test systems. The object of this guidance document is not to specify a particular remedy screening method but rather to highlight those critical parameters which should be evaluated during the laboratory test.

The test should include controls to measure the impact of abiotic (non-biological) processes such as volatilization, sorption, and photodecomposition on the concentrations of contaminants. Inhibited controls can be established by using formaldehyde, mercuric chloride or sodium azide to inhibit microbiological activity. However, care should be exercised when selecting a sterilizing agent. For example, sodium azide can, under certain circumstances, promote spontaneous explosive reactions. Mercuric chloride complexes certain petroleum hydrocarbons and results in artificially low hydrocarbon concentrations. Soil structure can also be modified by sterilization agents.

Complete sterilization of soils can be difficult to accomplish. Incomplete mixing of sterilization agents with soils can result in pockets of surviving microbes in soil pores. In some cases, microbial populations can transform and detoxify sterilizing agents. Complete sterilization of the control is not necessary, provided that biological activity is inhibited sufficiently so that a statistically significant difference between the test and control means can be determined. However, care should be taken in interpreting remedy screening study results. Substantial degradation In the control (e.g., 20-50% contaminant reduction, or more) can mask the fact that biodegradation occurred in the test reactor. If the control reactor has the same or greater percent degradation as the test reactor, a false negative conclusion can result. Concluding that no biodegradation occurred, when in fact there was some biodegradation, can lead to elimination of this technology unnecessarily. Alternatively, closed test systems with volatile traps can be used to monitor the volatilization of compounds instead of using inhibited controls to estimate abiotic losses.

A statistical experimental design should be used to conduct the treatability study in order to support decisions made from the treatability data. The various parameters of interest are included as factors in the experimental design. The treatability experiment should include monitoring the concentration of chemicals of interest overtime. In general, at least 3 to 4 time periods should be studied, including the time-zero (T_o) analysis. However, if the study goals are met after a sampling period, then it is not necessary to continue sampling at additional time periods. (For example, if 70% reduction was achieved after one week, it would not be necessary to continue testing if the goal was only to achieve 20% reduction.)

The test system can consist of a single large reactor or multiple small reactors. In the case of the single reactor, small subsamples are removed at various times and compared to subsamples from a second reactor in which biological activity has been inhibited. Normally, triplicate subsamples are taken at each time point. The mean contaminant concentration in the inhibited control subsample is compared to that in the test subsample to determine whether statistically significant biodegradation of contaminant occurred at each time point. In this type of system, heterogeneity within the soil system can lead to variability in contaminant concentration among the various subsamples and replicates. However, such system variability can be overcome by thorough mixing of the soil before it is distributed to the test and control systems. Care must be taken to minimize the release of volatiles during mixing. Examples of this type of system are large flasks, soil pans and other large soil reactors. Care should be taken so that the system size and design do not limit the availability of oxygen and moisture and cause variability in degradation rates within the reactor.

Multiple reactors may be set up in place of a large soil system. Triplicate reactors are established for each test reactor and control group at each time point. Each reactor is filled with the same amount of soil and nutrient additives. In this case, the complete reactor contents are extracted and analyzed for each of the triplicate test and control reactors at each time point. Examples of such systems are serum bottles, slurry reactors and aerated soil reactors. The advantage of this type of experimental apparatus is that the question of subsampling representativeness is avoided. However, the representativeness of any one reactor is questionable in this design. Thorough mixing of the soil, before it is distributed among the individual reactors, is important.

Respirometric measurements or other measures of biological activity can be used to predict the best times to take samples. At the beginning of the experiment, activity measurements should indicate minimal biological activity. Con-

tinued monitoring can reveal either a rapid or relatively slow onset of biological activity, and give a good indication of when samples should be taken to monitor contaminant reductions. However, respirometric measurements can indicate the loss of oxygen through chemical oxidation in addition to biodegradation.

In formulating an experimental design, the total number of samples taken depends on the desired difference in concentrations that the experimenter wishes to detect, the measurement variability (the analytical coefficient of variation), and the statistical error probabilities.

SAMPLING AND ANALYSIS PLAN

The Sampling and Analysis Plan (SAP) consists of two parts–the Field Sampling Plan (FSP) and the Quality Assurance Project Plan (QAPjP). A SAP is required for all field activities conducted during the RI/FS. The purpose of the SAP is to ensure that samples obtained for characterization and testing are representative and that the quality of the analytical data generated is known. The SAP addresses field sampling, waste characterization, and sampling and analysis of the treated wastes and residuals from the testing apparatus or treatment unit. The SAP is usually prepared after Work Plan approval.

Field Sampling Plan

The FSP component of the SAP describes the sampling objectives; the type, location and number of samples to be collected; the sample numbering system; the necessary equipment and procedures for collecting the samples; the sample chain-of-custody procedures; and the required packaging, labeling and shipping procedures.

Field samples are taken to provide baseline contaminant concentrations and material for the treatability studies. The sampling objectives must be consistent with the treatability test objectives. Because the primary objective of remedy screening studies is to provide a first-cut evaluation of the extent to which specific chemicals are removed from the soil by biological process, the primary sampling objectives should include, in general:

- Acquisition of samples representative of conditions typical of the entire site or defined areas within the site. Because this is a first-cut evaluation, elaborate statistically designed field sampling plans may not be required. Professional judgment regarding the sampling locations should be exercised to select sampling sites that are typical of the area (pit, lagoon, etc.) or appear above the average concentration of contaminants in the area being considered for the treatability test. This may be difficult because reliable site characterization data may not be available early in the remedial investigation.

- Acquisition of sufficient sample volumes necessary for testing, analysis, and quality assurance and quality control.

Quality Assurance Project Plan

The Quality Assurance Project Plan should be consistent with the overall objectives of the treatability study. At the remedy screening level the QAPjP should not be overly detailed.

The intended purpose of this study is to determine if the concentration of the target compounds decreases at least 20% in the biological reactor compared to the inhibited control at an 80% confidence level. Only the relative accuracy of the analytical measurements and the overall precision of the experiments are important. The suggested QC approach will consist of:

- Triplicate samples of both reactor and inhibited control at each sampling time

- The analysis of surrogate spike compounds in each sample

- The extraction and analysis of a method blank with each set of samples

- The analysis of a matrix spike in approximately 10 percent of the samples.

The analysis of triplicate samples provides for the overall precision measurements that are necessary to determine whether the difference is significant at the 80 percent confidence level. The analysis of the surrogate spike will determine if the analytical method performance is consistent (relatively accurate). The matrix spike will be used to measure overall analytical accuracy. The method blank will show if laboratory contamination has had an effect on the analytical results.

Selection of appropriate surrogate compounds will depend on the target compounds identified in the soil and the analytical methods selected for the analysis.

TREATABILITY DATA INTERPRETATION

The information and results gathered from the remedy screening are used to determine if bioremediation is a viable treatment option and to determine if additional remedy selection and remedy design studies are needed prior to the implementation of a full-scale bioremediation process. A threshold of greater than 20% reduction in the concentrations of the compounds of concern, compared to the abiotic control, indicates that bioremediation is potentially a viable cleanup method and further testing is warranted. For some compounds or sites, a period of time longer than the typical 6-8 weeks may be indicative of a successful remedy screening study. An example method for interpreting the results from a remedy screening treatability study is provided below in Example 1. Other specifically valid statistical methods may be used as appropriate.

If the remedy screening indicates that bioremediation is a potential cleanup option then remedy selection studies should be performed.

Example 1.

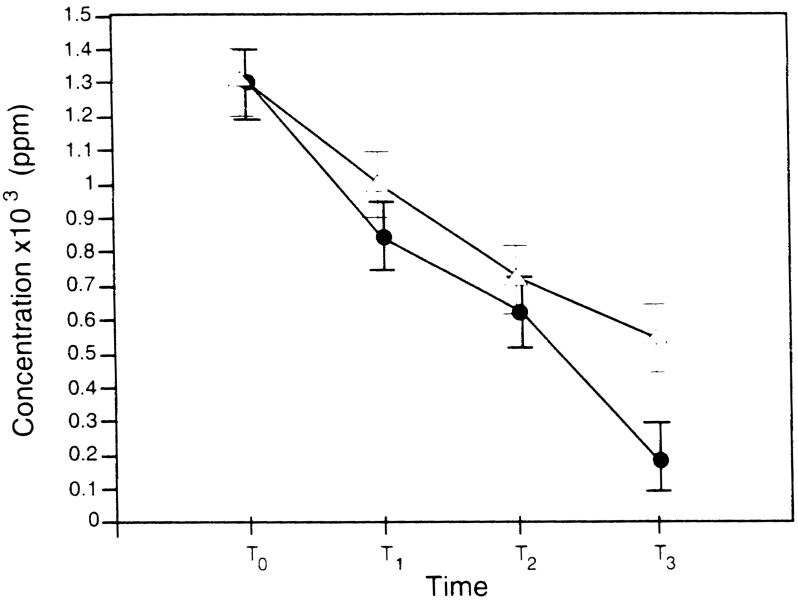
In a remedy screening treatability study for soil contaminated with a solvent, the average solvent concentrations in both the inhibited control and in the biologically active system were 1300 ppm at T_o. The average solvent concentration in the inhibited control was reduced to 550 ppm (T_3), a reduction of greater than 57 percent (Table 6-1). The average hydrocarbon concentration in the biologically active system was reduced to 200 ppm (T_3), a reduction of greater than 84 percent for the same time period.

TABLE 6-1. Hydrocarbon Concentration (ppm) Versus Time

SAMPLE	T_0	T_1	T_2	T_3
Inhibited Control (C_i)				
Replicate 1	1220	1090	695	575
Replicate 2	1300	854	780	580
Replicate 3	1380	1056	688	495
Mean Value	1300 ($\widehat{Ci}_0$)	1000 ($\widehat{Ci}_1$)	721 ($\widehat{Ci}_2$)	550 ($\widehat{Ci}_3$)
Concentration Change ($\widehat{Ci}_0 - \widehat{Ci}_t$) (T = 0, 1, 2, 3)	0	- 300	- 579	- 750
Bioreactor (C_b)				
Replicate 1	1327	982	550	225
Replicate 2	1320	865	674	310
Replicate 3	1253	703	666	65
Mean Value	1300 ($\widehat{Cb}_0$)	850 ($\widehat{Cb}_1$)	630 ($\widehat{Cb}_2$)	200 ($\widehat{Cb}_3$)
Concentration Decrease ($\widehat{Cb}_0 - \widehat{Cb}_t$) (T = 0, 1, 2, 3)	0	- 450	- 670	- 1100

The average contaminant concentration of the bioreactor, at each time point, is corrected by the average contaminant concentration of the inhibited control, at the same time point, to measure the biodegradation at that time point. The inhibited control accounts for contaminant losses due to volatilization, adsorption to soil particles, and chemical reactions. Some contaminant loss in the control due to biodegradation may occur since total sterilization is difficult to accomplish. However, if a statistically significant difference between the test and control means exists, then biodegradation has occurred in the test bioreactor. The difference between the two means is tested using Analysis of Variance (ANOVA) methods at the 80 percent confidence level for each of the test times. If the difference between the two means is significant at T_1, no further test measurements are required. If the difference between the two means is not significant at T_1, then the remedy screening test continues until some T_2. This process is repeated until a statistically significant difference between the two means is found or the treatability study is determined to be unsuccessful and is discontinued. In this example, a statistically significant difference between the two means occurs at T_3. The data, therefore, indicate that bioremediation is a viable treatment option and that further remedy selection studies are appropriate. The 80% confidence interval about each mean is shown in Figure 6-1 to graphically describe the variation associated with each mean.

Figure 6-1. Plot of hydrocarbon concentration versus time.



TECHNICAL ASSISTANCE

Literature information and consultation with experts are critical factors in determining the need for and ensuring the usefulness of treatability studies. A reference list of sources on treatability studies is provided in the "Guide for Conducting Treatability Studies Under CERCLA" (EPA/540/2-89-058).

It is recommended that a Technical Advisory Committee (TAC) be used. This committee includes experts of the technology who provide technical support from the scoping phase of the treatability study through data evaluation. Members of the TAC may include representatives from EPA (Region and/or ORD), other Federal Agencies, States, and consulting firms.

OSWER/ORD operate the Technical Support Project (TSP) which provides assistance in the planning, performance, and/or review of treatability studies. For further information on treatability study support or the TSP, please contact:

Groundwater Fate and Transport
Technical Support Center
Robert S. Kerr Environmental Research
Laboratory (RSKERL), Ada, OK
Contact: Don Draper
FTS 743-2200 or (405) 332-8800

Engineering Technical Support Center
Risk Reduction Engineering Laboratory
(RREL), Cincinnati, OH
Contact: Ben Blaney
FTS 684-7406 or (513) 569-7406

FOR FURTHER INFORMATION

In addition to the contacts identified above, the appropriate Regional Coordinator for each Region located in the Hazardous Site Control Division/Office of Emergency and Reme dial Response or the CERCLA Enforcement Division/Office of Waste Programs Enforcement should be contacted for additional information or assistance.

ACKNOWLEDGEMENTS

The fact sheet and the corresponding guidance document were prepared for the U.S. Environmental Protection Agency, Office of Research and Development (ORD), Risk Reduction Engineering Laboratory (RREL), Cincinnati, Ohio by Science Applications International Corporation (SAIC) under Contract No. 68-C8-0061. Mr. Dave Smith served as the EPA Technical Project Monitor, Mr. Jim Rawe served as the primary technical author and SAIC's Work Assignment Manager. Mr. Derek Ross (ERM) served as a technical expert. The author is especially grateful to Mr. Steve Safferman of EPA, RREL who contributed significantly by serving as a technical consultant during the development of this document.

Many other Agency and independent reviewers have contributed their time and comments by participating in the expert review meetings and/or peer reviewing the guidance document.